control (Oligo ASM, 5'-UGTCACCCTTTTTCATUCAC-3'; SEQ ID NO:50) were synthesized, purified, and analyzed as previously described (Agrawal S. et al., *Proc Natl Acad Sci USA*, **94**: 2620-2625, 1997). Two nucleosides at 5'-end and four nucleosides at 3'-end are 2'-O-methylribonucleosides (represented by boldface letters); the remaining are deoxynucleosides. The underlined nucleosides of Oligo ASM are the sites of the mismatched controls compared with Oligo AS. For both mixed-backbone oligonucleotides, all internucleotide linkages are phosphorothioate. The purity of the oligonucleotides were shown to be greater than 90% by capillary gel electrophoresis and PAGE, with the remainder being n-1 and n-2 products. The integrity of the internucleotide linkages was confirmed by ^{31}P NMR.

Please replace Table 7 on page 62 with the following table:

Name	SEQ ID NO:	Sequence
AS5-2	36	5′-TGA CAC CTG TTC TCA CTC AC-3′
AS5-2H	47	5′-UGA CAC CTG TTC TCA CUC AC-3′
AS5-2HM	48	5′-UGA GAC CAG TTG TCA GUC AC-3′

REMARKS

A. Preliminary Remarks

1. Amendments

The Applicants have made the foregoing amendments to the specification of the above-identified application (the "Specification") in order to correct typographical errors and to add sequence identifiers to certain nucleotide sequences in compliance with 37 C.F.R. § 1.821. The Applicants note that the sequence of the mismatched control Oligo M4, corrected by the amendment to the paragraph that spans page 4, line 15, to page 5, line 10, is supported by the specification as originally filed at page 31, line 23. In view of the foregoing, the Applicants respectfully submit that no new matter has been added.

2. Defective Oath or Declaration

As noted by the Examiner, the declarations filed August 15, 2000 were defective for incorrectly claiming priority from Application No. 08/916,834 instead of Application No. 08/916,384 (the "'384 Application"). The Applicants will provide fully executed Supplemental Declarations claiming correct priority.

B. Patentability Arguments

1. Double Patenting

a. The obviousness-type double patenting rejection should be staid.

Claims 1-29 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 16-43 of copending Application No. 09/383,507. Because this is a provisional rejection, in light of the fact that the conflicting claims have not in fact been patented, the Applicants will consider the possibility of filing a terminal disclaimer upon the allowance of the '507 Application (the "'507 Application").

2. Rejections Under 35 U.S.C. §102

a. The rejection of claim 10 under 35 U.S.C. §102(b) as being anticipated by WO 93/20238 should be withdrawn.

Claim 10 was rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by WO 93/20238 (the "'238 Application"). The Applicants respectfully traverse the rejection on the grounds that the subject matter of claim 10 is patentably distinguishable from the subject matter described in the '238 Application.

The Examiner characterized claim 10 as being drawn to a method of activating p53 in a tumor cell, comprising contacting said cells with an antisense oligo targeted to MDM2. In rejecting claim 10 under 35 U.S.C. § 102(b), the Examiner characterized the '238 Application as allegedly teaching that MDM2 can be inhibited by antisense oligos thereby allowing p53-regulated apoptosis.

The Applicants respectfully submit that the Examiner has incorrectly characterized the subject matter of the '238 Application. While the '238 Application mentions that MDM2 expression can be down-regulated using antisense oligonucleotides, the '238 Application does not explicitly teach that antisense oligonucleotides targeted to MDM2 leads to an activation of

p53 in tumor cells. In view of the '238 Application not explicitly disclosing that MDM2 antisense oligonucleotides activate p53 in tumor cells, claim 10 is patentably distinguishable from the '238 Application. Therefore, the Applicants respectfully submit that the rejection of claim 10 under 35 U.S.C. § 102(b) as being anticipated by the '238 Application is improper and should be withdrawn.

b. The rejection of claims 10 and 11 under 35 U.S.C. §102(b) as being anticipated by Kondo *et al.* should be withdrawn.

Claims 10 and 11 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Kondo *et al.* ("Kondo"). The Applicants respectfully traverse the rejection on the grounds that the subject matter of claims 10 and 11 is patentably distinguishable from Kondo.

The Examiner characterized the subject matter of claims 10 and 11 as being drawn to a method of activating p53 in a cell or enhancing DNA damage-induced activation of p53 in tumor cells, comprising contacting said cells with a DNA damaging agent and an antisense oligo targeted to MDM2. In rejecting claims 10 and 11 under 35 U.S.C. § 102(b), the Examiner characterized Kondo as disclosing the administration of MDM2 antisense oligos to glioblastoma cells, wherein the cells were also treated with the DNA damaging agent cisplatin, causing an increase in p53 expression.

The Applicants respectfully submit that the Examiner has incorrectly characterized the subject matter disclosed in Kondo. While Kondo does mention that exposure of glioblastoma cells to cisplatin induces the expression of p53, Kondo does not teach that exposure of glioblastoma cells to either (i) MDM2 antisense oligos alone (subject matter of claim 10) or (ii) MDM2 antisense oligos together with cisplatin (subject matter of claim 11), induces the expression of p53. In fact, Kondo states the following:

> [N]either mdm2 transfection nor mdm2 antisense treatment affected the levels of wild-type p53 protein in cisplatin-treated tumor cells.

See Kondo, page 2003, left-hand column, and Figure 5A. Based on that citation, the Applicants respectfully submit that Kondo actually teaches away from the subject matter of claims 10 and 11. In contrast to Kondo, the Specification discloses that the treatment of tumor cells with the combination of MDM2 antisense oligonucleotides and DNA damaging agent camptothecin causes a synergistic activation of p53. *See* Example 8. In view of the failure of Kondo to show

activation of p53 expression upon exposure of tumor cells to MDM2 antisense oligonucleotides, with or without a DNA damaging agent, the Applicants respectfully submit that the rejection of claims 10 and 11 under 35 U.S.C. § 102(b) as being anticipated by Kondo is improper and should be withdrawn.

3. Rejections Under 35 U.S.C. §103

a. The rejection of claims 10-12 under 35 U.S.C. §103(a) as being unpatentable over Kondo *et al.* in view of Clark *et al.* should be withdrawn.

Claims 10-12 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kondo in view of Clark *et al.* (hereinafter, "Clark"). The Applicants traverse the rejection on the grounds that the combination of Kondo and Clark does not teach or suggest the subject matter of claims 10-12.

The Examiner characterized the subject matter of claims 10-12 as being drawn to a method of enhancing DNA damage-induced activation of p53 in tumor cells, comprising contacting said cells with camptothecin and an antisense oligo targeted to MDM2. In rejecting claims 10-12 under 35 U.S.C. § 103(a), the Examiner relied on the teachings of Kondo discussed above in the rejection under 35 U.S.C. § 102(b) and noted that Kondo failed to teach the administration of camptothecin as the DNA damaging agent. The Examiner characterized Clark as teaching the administration of camptothecin in the treatment of pancreatic cancer. The Examiner concluded that it would have been obvious to one of ordinary skill in the art to use camptothecin in lieu of cisplatin in the methods of Kondo since both agents are recognized as DNA damaging agents and are thus able to be used interchangeably. The Examiner further concluded that one would have had a reasonable expectation of success of using camptothecin since both camptothecin and cisplatin are anti-cancer drugs. Based on the conclusions above, the Examiner alleged that claims 10-12 would have been *prima facie* obvious to one of ordinary skill in the art over Kondo in view of Clark.

Once again, the Applicants respectfully submit that the Examiner has incorrectly characterized Kondo. As described above, Kondo fails to demonstrate that the administration of MDM2 antisense oligonucleotides to tumor cells activates the expression of p53, with or without a DNA damaging agent. The fact that Clark teaches the use of camptothecin to treat pancreatic cancer does nothing to overcome the above-mentioned shortcomings of Kondo, nor does Clark provide any additional suggestion that DNA damage can be synergistically enhanced by inducing

the activation of p53 expression in tumor cells with MDM2 antisense molecules, as is taught by Example 8 of the Specification.

In view of the failure of the combination of Kondo and Clark to teach or suggest that DNA damage-induced synergistic activation of p53 in tumor cells is enhanced by administering camptothecin and MDM2 antisense oligonucleotides, the Applicants respectfully submit that the rejection of claims 10-12 under 35 U.S.C. § 103(a) as being unpatentable over Kondo in view of Clark is improper and should be withdrawn.

The Applicants respectfully submit that the rejection of claims 11 and 12 under 35 U.S.C. § 103(a) is also improper and should be withdrawn because claim 10, from which claims 11 and 12 depend, satisfies the requirements of nonobviousness under 35 U.S.C. § 103. *See In re Royka*, 490 F.2d 981, 180 USPQ 580 (C.C.P.A. 1974) (a dependent claim is nonobvious if the claim from which it depends is nonobvious under 35 U.S.C. § 103).

4. Rejections Under 35 U.S.C. §112

a. The rejection of claims 1-29 under 35 U.S.C. §112, first paragraph, for lack of enablement should be withdrawn.

Claims 1-29 were rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to enable one of ordinary skill commensurate in scope with said claims. The Examiner characterized the invention of claims 1-29 as being drawn to a method of inhibiting tumor growth by administering to a mammal (i) an MDM2 antisense oligo, or (ii) a combination of a chemotherapeutic and an MDM2 antisense oligo. The Examiner further characterized the invention as being drawn to administering antisense oligos targeted to SEQ ID NOS: 2-4, 7-11, and 13-24 of the MDM2 transcript as well as the antisense oligos comprising SEQ ID NOS: 27-46. The Examiner stated that the Specification is enabling for the *in vitro* administration of MDM2 antisense oligonucleotides. However, the Examiner rejected claims 1-29 under 35 U.S.C. § 112, first paragraph, for lack of enablement, alleging that the field of antisense therapy is unpredictable and without further examples, one of ordinary skill in the art would not accept the *in vivo* success of SEQ ID NOS: 28 or 47 as being correlative of the genus sought.

1) Predictability of Antisense Technology

The Examiner based the rejection of claims 1-29 under 35 U.S.C. § 112, first paragraph, for lack of enablement on various citations from Agrawal, Branch, and Gevirtz et al. (hereinafter, "Gevirtz") (collectively, the "References"), alleging that the Specification is not enabling *in vivo*

because antisense oligonucleotide therapy is unpredictable, controversial and unreliable. The Applicants respectfully traverse the rejection for nonenablement on the grounds that (i) the Examiner has incorrectly characterized the teachings of the References regarding the predictability of antisense therapy, and (ii) the Examiner has not considered the entire disclosure of the Specification.

a) Cellular Uptake of Oligonucleotides

The Examiner characterized Agrawal and Gevirtz as disclosing that the cellular uptake of oligonucleotides is unpredictable. As cited by the Examiner, Agrawal states the following:

> Oligonucleotides must be taken up by cells in order to be effective. Several reports have shown that efficient uptake of oligonucleotides occurs in a variety of cell lines, including primary cells, whereas other reports indicate negligible cellular uptake of oligonucleotides. Cellular uptake of oligonucleotides is a complex process; it depends on many factors, including the cell type, the stage of the cell cycle, the concentration of serum... It is, therefore, difficult to generalize that all oligonucleotides are taken up in all cells with the same efficiency.

See Agrawal, page 378. Although Agrawal discloses that the uptake of oligonucleotides in a particular *in vitro* cell culture system does not necessarily correlate to the uptake of oligonucleotides by other cells lines *in vitro*, the Applicants respectfully submit that the Specification provides sufficient detail to enable one of skill in the art to practice the invention.

The Specification discloses (i) the uptake and activity of 9 different MDM2 antisense oligonucleotides by 2 different tumor cells lines, and (ii) the uptake and activity of an MDM2 antisense oligonucleotide by 22 different tumor cells lines. *See* Examples 9 and 11. Furthermore, one of ordinary skill in the art could test additional cell lines for the *in vitro* uptake of oligonucleotides without undue experimentation using such routine methods as measuring the uptake of radiolabeled oligonucleotides. As for the uptake of oligonucleotides *in vivo*, the Applicants note that Agrawal cites at least eight examples confirming the uptake and activity of antisense oligonucleotides in mammalian *in vivo* models. *See* Agrawal, page 376. Based on the foregoing disclosure in the Specification and the knowledge of one of ordinary skill in the art, the Applicants respectfully submit that one of ordinary skill in the art would be able to readily determiner the effects of a change in the subject matter to which the invention pertains.

b) "Artificial Systems"

The Examiner also characterized Agrawal as disclosing that it is impossible to predict *in vivo* antisense activity based on antisense activity in artificial cell culture systems. The citation from Agrawal relied on by the Examiner is as follows:

> Another parameter that affects the potency and specificity of an oligonucleotide is the target gene itself. Some cell culture studies have been carried out using transfected cells, in which a segment of the gene of interest is inserted into a plasmid under the control of an appropriate promoter. In such studies, the rate of transcription, folding of RNA and the overall environment of the entire process is very different from the transcription and folding of the full-length gene of interest. In addition, the segment of the gene alone may or may not have a crucial function. *Any antisense activity observed in such artificial systems should be scrutinized carefully with respect to the disease process and its applicability to in vivo applications.*

See Agrawal, page 379 (emphasis added to portion quoted by the Examiner at page 8 of the Office Action). The Applicants respectfully submit that the Examiner has mischaracterized the scope of the foregoing disclosure in Agrawal, by characterizing "artificial systems" as referring to "artificial systems (cell culture)." *See* Office Action, page 8. The Applicants note that the "artificial systems" referred to by Agrawal are cell culture studies where the cell lines do not normally express the target gene (i.e., the cell lines have been transfected with the target gene). Furthermore, the "artificial systems" referred to by Agrawal are cell culture studies where the target mRNA is transcribed from only a portion of the gene of interest (i.e., the target gene is not full length). Using such "artificial systems" where both (i) the mechanism for transcribing the target gene and (ii) the primary and secondary structure of the target mRNA are different from the conditions *in vivo* are situations where the applicability of *in vitro* antisense activity to *in vivo* applications "should be scrutinized carefully." By contrast, each of the cell culture studies disclosed in the Specification were performed using only cell lines that naturally contain full-length genes for p53 and MDM2. In view of the Specification disclosing cell culture studies using cell lines that naturally contain full length copies of the gene for p53 and MDM2, the Applicants respectfully submit that the unpredictability of antisense activity in "artificial" *in vitro* systems is not applicable to the claimed invention.

c) Animal Models

The Examiner failed to note that Agrawal concludes that due to the unpredictability of antisense activity in the "artificial systems" described above, animal models may be necessary to confirming the antisense effects of oligonucleotides in tissue culture screening. *See* Agrawal page 385, left-hand column. As mentioned above, the cell culture studies disclosed in the Specification do not have the inherent problems of the "artificial systems" described by Agrawal. Nevertheless, the Applicants have confirmed the antisense activity of MDM2 antisense oligonucleotides by showing that MDM2 antisense oligonucleotides corresponding to SEQ ID NOS: 28 and 47 inhibit the growth of tumors *in vivo*. *See* Examples 16 and 17. In view of the confirmation of antisense activity *in vivo*, the Applicants respectfully submit that the Specification demonstrates that oligonucleotides with MDM2 antisense activity in the cell culture systems disclosed in Example 1-15 correlates to *in vivo* MDM2 antisense activity.

d) Non-Antisense Effects

The Examiner characterized Branch and Gevirtz as disclosing that antisense therapy is unpredictable *in vivo* because of non-antisense effects. Branch discloses that quantitative dosage information is a means of showing that activity of antisense oligonucleotides is not due to non-antisense effects. Branch and Gevirtz disclose that non-antisense effects are a problem with many antisense oligonuceotides because the target site of the mRNA transcript is often inaccessible due to the native structure of the transcript and binding of the transcript to cellular proteins. As cited by the Examiner, Branch concludes that "[b]ecause non-antisense effects are not currently predictable, ... [t]hese effects must be explored on a case-by-case basis." The Applicants acknowledge that the accessibility of target cites on the mRNA transcript of a target gene require experimentation to determine the activity of antisense oligonucleotides. However, the Applicants respectfully submit that the Specification discloses methods which enable one of ordinary skill in the art to identify MDM2 antisense oligonucleotides with antisense activity.

As mentioned above, the Specification discloses the use of cell culture systems where the mechanism for transcribing the MDM2 target gene is the same as *in vivo.* Furthermore, the cell culture systems disclosed in the Specification produce an MDM2 target gene transcript with the same the same primary and secondary structure present *in vivo.* Therefore, the conditions of the MDM2 target gene transcript in the cell culture systems disclosed in Examples 1-15 are the same

as the conditions present *in vivo.* As a result, the activity of MDM2 antisense oligonucleotides in the disclosed cell culture systems is not due to non-antisense effects. Moreover, the Specification confirms that the activity of the MDM2 antisense oligonucleotides is due to specific antisense activity, because the Applicants show that MDM2 antisense oligonucleotides decrease intracellular levels of MDM2 protein, . *See* Example 1 and Figure 2A. As a final confirmation that antisense activity in the cell culture system is indicative of antisense activity *in vivo,* the Specification discloses that oligonucleotides corresponding to SEQ ID NOS: 28 and 47 have antisense activity in the cell culture system and *in vivo*. *See* Example 16 and 17.

e) Standards in the Art

The Examiner failed to note that Branch discloses that the unpredictability of antisense results, under certain experimental conditions, led the editors of *Antisense and Nucleic Acid Drug Development* to publish standards for conducting antisense studies. *See* Branch, page 47, first column. Under the revised standard of review, manuscripts submitted to *Antisense and Nucleic Acid Drug Development* for review claiming antisense activity must (i) demonstrate a decrease in the levels of the target protein, and (ii) conduct experiments with at least two types of control oligonucleotides. *See Stein and Krieg*, page 68 (attached as Appendix B). As mentioned above, Example 1 and Figure 2A of the pending application demonstrate that the expression of MDM2 protein is inhibited by MDM2 antisense oligonucleotides. Furthermore, the Applicants conducted the studies described in the specification using a sense control (oligonucleotide K) and a mismatched control (oligonucleotides M4, AS2M2, AS2M4, AS5M4 or As5-2HM). In view of the Applicants demonstrating antisense activity in a manner that satisifies the standards set by a peer reviewed journal in the art of antisense technology, the Applicants respectfully submit that one of ordinary skill in the art would reasonably expect that MDM2 antisense oligonucleotides with activity in the cell culture systems of Examples 1-15 would be due to specific antisense effects.

f) Reasonable Correlation

The Applicants do acknowledge some of the general difficulties associated with antisense therapy described by Agrawal, Gervitz and Branch. However, the Applicants submit that the Specification provides sufficient guidance to a person of ordinary skill in the art to allow the use of any of the full range of oligonucleotides claimed, without undue experimentation, because

MDM2 antisense oligonucleotides with activity in the cell culture systems described in the Specification correlate to *in vivo* antisense activity.

The Examiner alleged that there is no correlation between the *in vivo* activity of SEQ ID NOS: 28 or 47 and the *in vitro* activity of the disclosed MDM2 antisense oligonucleotides. The Applicants note that only a "reasonable correlation is required between the disclosed *in vitro* utility and an *in vivo* activity." *See Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q.2d 739 (Fed. Cir. 1985) (emphasis added). The Applicants respectfully submit that the Specification discloses a "reasonable correlation" between the *in vitro* utility and *in vivo* activity of MDM2 antisense oligonucleotides because the Applicants have demonstrated that MDM2 antisense oligonucleotides which effectively induce p53 activity in tumor cell lines also inhibit tumor growth in mice. See Examples 16 and 17. Furthermore, as described above, Examples 16 and 17, and the controls therein, meet the standards of review for *Antisense and Nucleic Acid Drug Development*. Therefore, the Applicants respectfully submit that the disclosure in the Specification would lead one of ordinary skill in the art to reasonably expect that MDM2 antisense oligonucleotides with *in vitro* activity would correlate to *in vivo* antisense activity.

2) *In re Wands*

In addition to the comments above, the Applicants respectfully traverse the rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement, on the grounds that the Examiner has failed to consider all of the factors for enablement set forth in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). The factors set forth in *Wands* for the consideration of enablement are as follows:

i) breadth of the claims;
ii) nature of the invention;
iii) state of the prior art;
iv) level of one of ordinary skill in the art;
v) level of predictability in the art;
vi) amount of direction provided by the inventor;
vii) existence of working examples; and
viii) quantity of experimentation needed to make or use invention based on the content of the disclosure.

The Applicants respectfully submit that based on the above-cited factors, claims 1-29 are enabling to one of ordinary skill in the art. Furthermore, the Applicants respectfully submit that the application of the *Wands* factors in *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 52

U.S.P.Q.2d 1129 (Fed. Cir. 1999) is applicable to the present application. The patents at issue in *Enzo* taught the application of antisense technology in the regulation of three *E. coli.* genes, while the claims (the "Enzo Claims") encompassed the broad application of antisense technology in all prokaryotes and eukaryotes. Based on a consideration of the Wands factors, the Federal Circuit affirmed the decision of the district court that certain patents were invalid (the "Enzo Patents") because the breadth of enablement in the specifications was not commensurate in scope with the claims.

With respect to "level of predictability in the art," the Applicants refer to the above discussion of the predictability of antisense technology. With respect to "breadth of claims," the Federal Circuit found the Enzo Claims to be extraordinarily broad, because an infinite number of genes and cell types were encompassed. *See Enzo* at 1372. By contrast, claims 1-29 are significantly limited because the claims are drawn to a *single* mammalian gene - MDM2.

The Federal Circuit found that the "amount of direction provided by the inventor" and "existence of working examples" present in the Enzo Patents was extremely narrow despite the broad breadth of claims because "virtually no guidance, direction, or working examples were provided for practicing the invention in eukaryotes, or even any prokaryote other than the three genes in *E. coli.*" *See Id.* at 1375. By contrast, the Specification gives specific guidance for the *in vitro* identification and optimization of antisense oligonucleotides with activity towards the claimed target gene, thereby providing direction and working examples for practicing the invention.

The Federal Circuit found that the "quantity of experimentation needed to make or use invention based on the content of the disclosure" of the Enzo Patents was quite high because the inventors failed to demonstrate antisense activity in eukaryotes or in prokaryotes other than *E. coli*. *See Enzo* at 1374. The Applicants acknowledge that experimentation will be required to practice the invention encompassed by claims 1-29; however, the required experimentation will not be "undue" because the pending application sets forth *in vitro* methods for identifying and optimizing MDM2 antisense oligonucleotides capable of increasing p53 expression. Furthermore, the *in vivo* inhibition of tumor growth by MDM2 antisense oligonucleotides in Examples 16 and 17 indicates that *in vitro* activity correlates with *in vivo* activity and a reasonable likelihood of success.

With respect to "state of the prior art" and "level of one of ordinary skill in the art," the Applicants respectfully submit that both (i) the state of the art and (ii) the level of one of ordinary skill in the art, have advanced significantly between 1983 (earliest priority date of the Enzo Patents) and 1997 (earliest priority date of the pending application).

The Federal Circuit summarized its review of the Enzo Patents by stating:

> What is glaringly "missing" from the specifications is the disclosure of any direction or examples of how [antisense technology] might be implemented in any cell other than *E. coli*. [Patentee's] disclosure of practicing antisense in *E. coli* does not suffice to enable the practice of antisense in all categories of living matter.

See Enzo at 1375. The Specification is not "missing" what the Federal Circuit referenced in the Enzo Patents, because the Specification in fact discloses antisense activity in the claimed "categor[y] of living matter" by showing antisense activity in mammalian cell lines and antisense activity in a mouse tumor model. In traversing the rejection for lack of enablement, the Applicants respectfully submit that while the Examiner's comments regarding lack of enablement are appropriate for the Enzo Patents, they are not appropriate for claims 1-29 of the pending application. Based on the foregoing comments, the Applicants respectfully submit that the full scope of the invention encompassed by claims 1-29 is enabled and therefore that the rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

CONCLUSION

In view of the above remarks, the Applicant respectfully submits that the instant application is in good and proper order for allowance. Early notification of this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite this prosecution of the instant application, the Examiner is encouraged to call the undersigned at (312) 902-5464.

Respectfully submitted,

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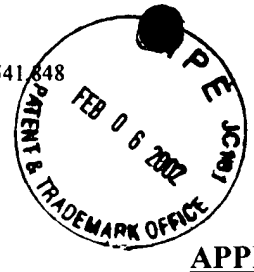

APPENDIX A TO AMENDMENT

Pursuant to 37 C.F.R. §1.121(c)(1)(ii), Applicant presents herewith marked-up text of the paragraphs of this application as amended by the foregoing amendment.

The paragraph that spans page 4, line 15, to page 5, line 10.

Recently, we have successfully identified an anti-MDM2 antisense PS-oligo that effectively inhibits MDM2 expression in tumor cells containing MDM2 gene amplifications (Chen L. et al., *Proc Natl Acad Sci USA*, **95**: 195-200, 1998). Effective anti-human-MDM2 antisense PS-oligos were initially screened in two cell lines, JAR (choriocarcinoma) and SJSA (osteosarcoma), that contain wild type p53, amplified MDM2 genes, and overexpression of MDM2 oncoprotein. Of nine PS-oligonucleotides screened, Oligo AS5 (5'GATCACTCCCACCTTCAAGG-3'; SEQ ID NO:28), which can hybridize to a position ~360 bp downstream of the translation start codon, was found to reproducibly decrease MDM2 protein levels in both cell lines by 3-5 fold at concentrations of 100-400 nM in the presence of Lipofectin. The mismatched control Oligo M4 [(5'-GATGACTCACACCATCAAGG-3')] (5'-GATGACTCACACCATCATGG-3'; SEQ ID NO:5) had no effect on MDM2 expression. Oligo AS5 was also shown to induce RNase H cleavage of the target MDM2 mRNA, resulting in truncation and degradation of the target. Further studies demonstrated that, following AS5 treatment, the p53 protein level was elevated and its activity was increased. A dose-dependent induction of p21 expression by AS5 was observed up to 6.6 fold at the optimal concentration of 200 nM, suggesting that p53 transcriptional activity be increased following inhibition of MDM2 expression. JAR cells treated with AS5 showed a significant increase in the levels of apoptosis. AS5 did not cause visible apoptosis in the H1299 cells that lack p53. These results suggested that apoptosis induced by AS5 [be] is due to activation of p53 following MDM2 inhibition by the oligonucleotide.

The paragraph that spans page 6, lines 10-20.

[p53-Independent] p53-independent activity of MDM2 has been suggested by several reports and reviews. MDM2 gene products include several forms of polypeptide, representing alternatively spliced MDM2 variants. Various alternatively spliced MDM2 polypeptides have been found in several human tumors. Of the five forms of MDM2 analogs, only one retains p53 binding capability. However, cDNAs coding for all five forms of alternatively spliced MDM2 could independently transform NIH3T3 cells, indicating that these MDM2 transcripts have the p53-independent transforming ability. The effects of MDM2 overexpression on mammary tumorigenicity are seen in p53-null mice, indicating that MDM2 can cause transformation and tumor formation via a p53-independent mechanism. More recently, overexpression of MDM2 is shown to be associated with resistance to the antiproliferative effects of transforming growth factor β (TGF-β), which is p53-independent.

The paragraph that spans page 10, line 8, to page 11, line 3.

In a fifth aspect, the invention provides *in vitro* and *in vivo* models to evaluate the therapeutic effectiveness of a recently identified anti-human-MDM2 antisense oligonucleotide (Chen L et al., *Mol Med* **5**: 21-34, 1999; Wang H. et al., Int *J. Oncol.* **15**: 653-660, 1999) in the treatment of human colorectal cancers when administered alone or in combination with conventional chemotherapeutic agents. Specifically, the primary goals are: 1) to obtain new oligos with better *in vivo* stability that can be used in future *in vivo* studies; 2) to determine the effects of anti-MDM2 oligos on human tumor cells with varying status of p53 and/or MDM2 expression; and 3) to identify the candidate cell lines that can be used in future *in vivo* studies. PS-oligonucleotide AS5-2 [(5'TGACACCTGTTCTCACTCAC-3')] (5'TGACACCTGTTCTCACTCAC-3'; SEQ ID NO:36) was shown to have the highest activity in tested cell lines and was used in further studies. Thus far, 26 cell lines (16 types of human cancers) have been tested with AS5-2 in comparison with control oligonucleotides. Oligo AS5-2 significantly activated p53 activity in all cells with low levels of wild type p53, even in those with very a low level of mdm2 expression (Chen L. et al., *Mol Med* **5**: 21-34, 1999). AS5-2 has no effect on p53 levels in cells with null p53, H1299 and SK-N-MC, or those with mutant p53. Based on the above screening, a modified analog of AS5-2 with advanced antisense chemistry, Oligo AS, was designed and evaluated in subsequent studies. In cell lines that contain wild type

p53 and amplified MDM2 gene, SJSA and JAR, Oligo AS specifically inhibits MDM2 expression and p53 levels are elevated accordingly (Wang H. et al., *Int J. Oncol.* **15**: 653-660, 1999).

The paragraph that spans page 21, lines 7-11.

In certain preferred embodiments, these internucleoside linkages may be phosphodiester, phosphotriester, phosphorothioate, or phosphoramidate linkages, or combinations thereof. The term oligonucleotide also encompasses such polymers having chemically modified bases or sugars [and/ or] and/or having additional substituents, including without limitation lipophilic groups, intercalating agents, diamines and adamantane.

The paragraph that spans page 22, lines 14-21.

For purposes of the invention, a "hybrid oligonucleotide" refers to an oligonucleotide having more than one type of nucleoside. One preferred embodiment of such a hybrid oligonucleotide comprises a ribonucleotide or 2′-O-substituted ribonucleotide region, preferably comprising from about 2 to about 12 2′-O-substituted nucleotides, and a deoxyribonucleotide region. Preferably, such a hybrid oligonucleotide will contain at least three consecutive deoxyribonucleosides and will also contain ribonucleosides, 2′-O-substituted ribonucleosides, or combinations thereof. Examples of such hybrid oligonucleotides are disclosed in U.S. [Patents] Patent Nos. 5,652,355 and 5,652,356.

The paragraph that spans page 47, line 18, to page 48, line 5.

Test Oligonucleotides. The test oligonucleotide, Oligo AS, a 20-mer mixed-backbone oligonucleotide (5'-UGACACCTGTTCTCACUCAC-3': SEQ ID NO. 47) and its mismatched control (Oligo ASM, 5'-UGTCACCCTTTTTCATUCAC-3': SEQ ID NO. 50) were synthesized, purified, and analyzed as previously described (Agrawal S. et al., *Proc Natl Acad Sci USA*, **94**: 2620-2625, 1997). Two nucleosides at 5'-end and four nucleosides at 3'-end are 2'-O-methylribonucleosides (represented by boldface letters); the remaining are deoxynucleosides.

The underlined nucleosides of Oligo ASM are the sites of the mismatched controls compared with Oligo AS. For both mixed-backbone oligonucleotides, all internucleotide linkages are phosphorothioate. The purity of the oligonucleotides were shown to be greater than 90% by capillary gel electrophoresis and PAGE, with the remainder being n-1 and n-2 products. The integrity of the internucleotide linkages was confirmed by ^{31}P NMR.

Table 7 on page 62.

Name	SEQ ID NO:	Sequence
AS5-2	[28] 36	5′-TGA CAC CTG TTC TCA CTC AC-3′
AS5-2H	47	5′-UGA CAC CTG TTC TCA CUC AC-3′
AS5-2HM	48	5′-UGA GAC CAG TTG TCA GUC AC-3′